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14. ABSTRACT Proposed vaccine principle relies on secreted gp96-Ig chaperoning PfCSP and PfAMA1 sporozoite proteins that are efficiently taken up and cross presented by activated DC via MHC I to CD8 CTL, thereby stimulating an avid, antigen specific, cytotoxic T cell response. This vaccine principle has been used successfully in murine models of cancer, in non-human primates for SIV vaccination and is in clinical trials for the treatment of non-small cell lung cancer patients. The generation of a powerful, cytotoxic anti sporozoite CD8 CTL response by the vaccine is expected to provide prophylactic immunity for malaria by removing infected liver cells before sporozoites can replicate and spread to the erythrocyte stage causing parasitemia. We establish collaboration with NMRC to design the vaccine cell lines. NMRC provided plasmid vectors carrying the PfAMA1 and PfCSP transgenes. We generated 293-gp96-Ig-PfAMA1-PfCSP cells by co-transfecting 293 cells with gp96-Ig, PfAMA1 and PfCSP. Completion of the 293-gp96-IgPfAMA1-PfCSP vaccine cell lines serves as the initial 'go', 'no-go' milestone for this application. We reached this milestone within the first year of the grant period, as it was proposed in SOW. We are currently expanding vaccine cells in order to proceed to proposed immunogenicity studies in mouse model.					
15. SUBJECT TERMS Malaria, Plasmodium Falciparum, circumsporozoite protein, apical membrane antigen-1, vaccine, heat shock proteins, gp96-Ig, cytotoxic T cells, cell mediated immunity					
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1. INTRODUCTION:

We have previously shown that cell-based vaccines secreting heat shock protein gp96-Ig (for short from here on: **gp96**) are safe for use in humans and represent the most efficient vaccine approach studied to date for stimulating multi-epitope specific cytotoxic T cells. In the proposed studies, we will adapt this vaccine approach to stimulate cytotoxic T cells against malaria antigens and investigate the optimal vaccination route to target these T cells to the liver. To accomplish these studies, we are collaborating with experts in the malaria vaccine field, Capt. Eileen F. Villasante, M.D., Ph.D., Head Malaria Department Infectious Diseases Directorate at Naval Medical Research Center. By conducting head-to-head studies to another promising malaria vaccine, these studies will help to set clinical priorities based on the most effective pre-clinical data in animal models.

2. KEYWORDS:

Malaria, *Plasmodium falciparum*, circumsporozoite protein, apical membrane antigen-1, vaccine, heat shock proteins, gp96-Ig, cytotoxic T cells, cell mediated immunity

3. OVERALL PROJECT SUMMARY:

The goal of our project is to combine the *Plasmodium falciparum* (Pf) antigens circumsporozoite protein (CSP) and apical membrane antigen-1 (AMA1) with a novel method of immunization that is based on the gp96-Ig vaccine platform to enable production of a strong, protective, cell-mediated immunity (CMI) response (interferon gamma [IFN- γ]-positive CD8+ cytotoxic T cells).

This will be accomplished through three specific aims: (1) construction of the 293-gp96-Ig^{PfAMA1-PfCSP} and 293^{PfAMA1-PfCSP} vaccine cell lines; (2) determination of the safety and immunogenicity of the 293-gp96-Ig^{PfAMA1-PfCSP} vaccine in mice; and (3) determination of the safety and immunogenicity of the 293-gp96-Ig^{PfAMA1-PfCSP} vaccine in rhesus macaques.

Summary of Current Objectives: During the last year we have been intensively working on the experiments related to **Specific Aim 1: Construction of the 293-gp96-Ig^{PfAMA1-PfCSP} and 293^{PfAMA1-PfCSP} vaccine cell lines.**

We establish collaboration with NMRC to design the vaccine cell lines. NMRC provided plasmid vectors carrying the PfAMA1 (VR2577) and PfCSP (VR2571) transgenes for use in transfecting HEK-293 cell lines with, and without gp96-Ig.

Summary of Results:

We transfected HEK-293 cells with gp96-Ig and with the *Plasmodium falciparum* circumsporozoite protein (PfCSP) and apical membrane antigen-1 (PfAMA1).

Codon optimized *Plasmodium* antigens were obtained from Capt. Villasante's research group at NMRC. Dr. Podack's team coordinated the molecular cloning and optimization of the *Plasmodium* antigens into appropriate mammalian expression vectors for transfection into HEK-293 cells together with gp96-Ig. Two-step transfection protocol was followed in order to establish stable triple transfected cell line. First, HEK-293 cells were transfected with the plasmid expressing gp96-Ig. After selecting for gp96-Ig expressing cells, we perform the second step of transfection with plasmids expressing PfAMA1 and PfCSP transgenes. Finally, we obtained the vaccine cells that consist of HEK-293 co-transfected with **gp96-Ig** and following malaria antigens **PfAMA1 (VR2577)** and **PfCSP (VR2571)**. Specifically, these respective cell lines were generated:

1. HEK-293 cells that secrete gp96-Ig
2. HEK-293 cells that secrete gp96-Ig and express PfAMA1
3. HEK-293 cells that secrete gp96-Ig and express PfCSP
4. HEK-293 cells that secrete gp96-Ig and express PfAMA1 + PfCSP
5. HEK-293 cells
6. HEK-293 cells that express PfAMA1
7. HEK-293 cells that express PfCSP
8. HEK-293 cells that express PfAMA1 + PfCSP

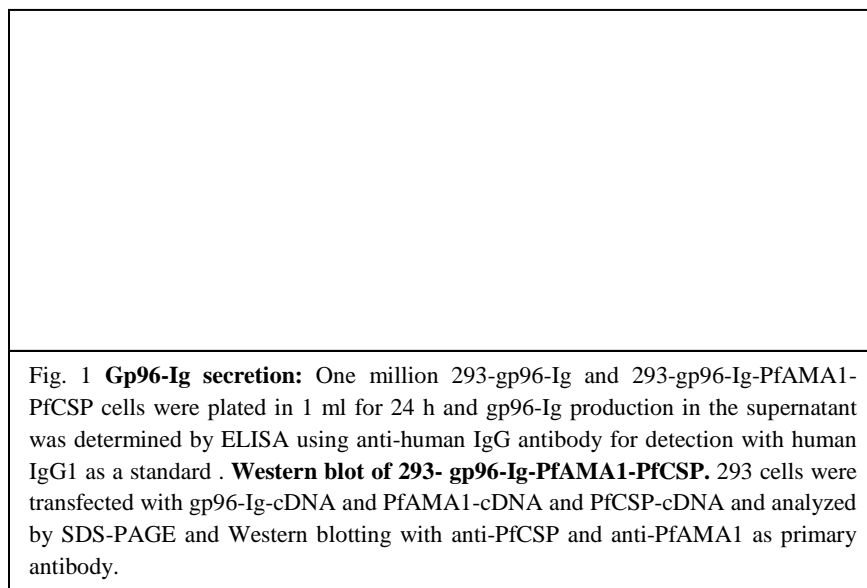


Fig. 1 **Gp96-Ig secretion:** One million 293-gp96-Ig and 293-gp96-Ig-PfAMA1-PfCSP cells were plated in 1 ml for 24 h and gp96-Ig production in the supernatant was determined by ELISA using anti-human IgG antibody for detection with human IgG1 as a standard . **Western blot of 293- gp96-Ig-PfAMA1-PfCSP.** 293 cells were transfected with gp96-Ig-cDNA and PfAMA1-cDNA and PfCSP-cDNA and analyzed by SDS-PAGE and Western blotting with anti-PfCSP and anti-PfAMA1 as primary antibody.

We characterized the secretion of gp96-Ig using the standardized ELISA protocol for human-IgG by plating 10^6 cells/ml and measuring the secreted gp96-Ig in the supernatant after 24 h culture. HEK-293-gp96-Ig-PfAMA1-PfCSP cells are

producing ~500 ng/ml (Figure 1). Stable transfected cell line (HEK-293-gp96-Ig) was subsequently co-transfected with plasmids expressing PfCSP (VR2571) and PfAMA1 (VR2577) anti-

gens or combination. We have confirmed the expression of PfCSP and PfAMA1 in the triple transfected HEK-293 cell line (HEK-293-gp96-Ig-PfAMA1-PfCSP) (Figure 1).

We are currently expanding all the cells and working on the production of the master cell bank that will be used for all mouse and non-human primate in vivo immunogenicity studies.

Summary of Progress and Accomplishment with Discussion:

Completion of the 293-gp96-IgPfAMA1-PfCSP vaccine cell lines serves as the initial 'go', 'no-go' milestone for this application.

We reached this milestone within the first year of the grant period, as it was proposed in SOW.

We demonstrated and characterized the secretion of gp96-Ig at a level of 500ng/24 hrs x 10^6 cells. Optimal CD8 CTL responses in mice are usually obtained by injecting the number of cells that secrete between 200 ng to 2 μ g gp96-Ig within 24 h (in culture). Similarly, we have performed dose-ranging studies in rhesus macaques, which demonstrated that the optimal quantity of secreted gp96-Ig for immunization in rhesus macaques is achieved between 5 and 10 μ g. Upon injection the cells are detectable for up to 5 days secreting gp96-Ig-peptide and continuously stimulate CD8 CTL responses over this period.

Following completion of this milestone, we will proceed immediately to Specific Aim 2 to determine the immunogenicity of these vaccines in mice.

4. KEY RESEARCH ACCOMPLISHMENTS:

- We demonstrated and characterized the secretion of gp96-Ig at a level of 500ng/24 hrs x 10^6 cells and expression of malaria antigens in 293-gp96-IgPfAMA1-PfCSP cell line.

5. CONCLUSION:

Our approach to vaccine development is to develop a multi-antigen malaria vaccine by generating high levels of multi-epitope, plasmodium-antigen specific CD8 cytotoxic T lymphocytes, mimicking the radiation attenuated whole parasite. Our experience documents that the cell based gp96-Ig approach is highly effective in generating high levels of antigen specific CD8 CTL which is effective in stimulating high-frequencies of poly-antigen specific CTL in both human cancer patients and SIV-specific CTL in rhesus macaques and which is safe. We adapted this vaccine strategy to malaria, and we transfect HEK-293 cells with the Plasmodium Falciparum circumsporozoite protein (PfCSP) and apical membrane antigen 1 (PfAMA-1) and with gp96-Ig and generated vaccine cells line 293-gp96-Ig^{PfAMA1-PfCSP}. Our studies are designed to enable a phase I clinical trial in humans by the end of the grant period and will provide a head-to-head comparison to another promising malarial vaccine candidate, NMRC-M3V-Ad-PfCA. The ultimate goal is to develop a universal vaccine that is highly effective and practical, which is in line with the DoD area of research interest. Our work in the next year will include studies that will determine safety and immunogenicity of the 293-gp96-Ig^{PfAMA1-PfCSP} vaccine in mice.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

Nothing to report

7. INVENTIONS, PATENTS AND LICENSES:

Nothing to report

8. REPORTABLE OUTCOMES:

Nothing to report

9. OTHER ACHIEVEMENTS:

- We have developed 293-gp96-IgPfAMA1-PfCSP cell line

10. REFERENCES:

Nothing to report

11. APPENDICES:

Nothing to report